RESPONSE OF HARD RED WINTER WHEAT (*Triticum aestivum* L.) TO PHOTOPERIOD AND VERNALIZATION IN SOUTH TEXAS

A Thesis

by

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ABSTRACT

The effects of vernalization and photoperiod on hard red winter wheat (*Triticum aestivum* L.; HRW) adaptation in the U.S. Great Plains is not well understood.

The main objectives of this study were to 1) characterize U.S. Great Plains HRW for vernalization requirement and photoperiod response and to 2) discern the association between the main effects of photoperiod (ΔP) and vernalization (ΔV) with yielding ability, plant height, days to heading, and vernalization and photoperiod marker data generated by the USDA-ARS Genotyping Laboratory in Manhattan, KS in 2010.

The Southern Regional Performance Nursery (SRPN) is a collection of experimental HRW lines from the Southern Region of the U.S. Great Plains. Of the 48 lines in the 2010 SRPN, 20 were selected for evaluation under growth chamber conditions at Texas A&M University in 2010, 2011, and 2012. Three photoperiod regimes were utilized to mimic short, optimum, and long day conditions (12 and 16 h of light in 2010 and 2011; 10, 14, and 16 h of light in 2012). Vernalized (6 wk; V; 2010, 2011, and 2012), moderately vernalized (3 wk; MV; 2012) and non-vernalized (0 wk, NV; 2010, 2011, and 2012) seedlings were transplanted into the different photoperiod regimes described above. Data was taken on days to head emergence and days to anthesis. Data compiled by the USDA-ARS at 30 field locations across the U.S. Great Plains was also utilized, including grain yield (ton ha^{-1}), yield stability (Eberthart and Russell stability parameters; β and δ^2), days to head emergence (d), and plant height (inch). Genotyping was done at the USDA-ARS genotyping lab in Manhattan, KS in

2010, using a K-Biosciences SNP pipeline and utilizing KASP chemistry. The Markers considered for this experiment were photoperiod marker PPD-D1 LD and markers for the three vernalization genes, *Vrn-A1, Vrn-A1b* and *Vrn-D3.* Basal vegetative period (BVP), a.k.a. intrinsic earliness, was measured as time for vernalized seedling to grow to anthesis in the long day photoperiod regime based on 2010 and 2011 evaluations. Furthermore, ΔV was measured as the difference in anthesis date between V and MV vernalization regimes under long day conditions, based on 2012 evaluation. Moreover, ΔP was measured as difference in days to anthesis between 16 and 12 h regimes in the vernalized seedlings based on 2010 and 2011 evaluations. There was a significant and positive correlation between ΔP and ΔV ($r=0.55$, $P < 0.05$). Our results also showed that taller and late-maturing HRW lines had larger ΔP and ΔV and were generally poor-yielding and less stable across environments. This was consistent in older HRW cultivars such 'Kharkof', 'Scout 66' and 'TAM 107'. Our study confirmed that HRW lines that yielded well across a broad geographic area were generally photoperiod-insensitive and had lower vernalization requirements. This combination also appeared to be vital for HRW lines adapted to South Texas climates.

NOMENCLATURE

HRS	Hard red spring wheat
HRW	Hard red winter wheat
SRPN	Southern regional performance nursery
SRW	Soft red winter wheat
USDA-ARS	U. S. Department of Agriculture-Agriculture research service

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CHAPTER I

INTRODUCTION

Wheat (*Triticum aestivum* L.) is grown in various environments throughout the world depending on rainfall totals and temperature ranges during the growing season (Stefany, 1993). It has been predicted that by 2050 there will be a 60% increase in demand for wheat to meet the growing population (Lucus, 2013). The United States (U.S.), being one of the major wheat producing countries, preceded only by China, the European Union and India, planted 22.72 million hectares of wheat in 2013. In the U.S., 70 to 80% of all wheat grown is winter wheat (*Triticum aestivaum* Desf.) as opposed to spring wheat (Vocke, 2013a). This is partially due to the fact that winter wheat has a higher yield potential than spring wheat because of a longer growing season. In order for spring wheat yields to compare with those of winter wheat it would require up to a 20% increase in nitrogen fertilizer (Vocke and Ali, 2013). In Texas, wheat producers were predicted to plant 2.3 million hectares of wheat in 2013 (Lewis and Johnson, 2013). The major wheat growing areas of Texas plant hard red winter wheat (HRW) with some exceptions being; soft red winter wheat (SRW) in East Texas and some hard red spring wheat (HRS) in South Texas. The HRS class is predominantly grown during the spring in the Northern plains of the U.S. and Canada. HRW is grown from Texas, throughout the Great Plains states and into Montana (Vocke, 2013b). Due to unpredictable and varied weather patterns in South Texas during the winter wheat growing season, it is challenging for producers to decide on which type of wheat will best fit their environment. Wheat production trends suggest that HRS production in Texas differs

from the northern U.S. and Canada, where it is typically grown as a summer crop, planted in early April and harvested in July (Vocke and Ali, 2013). Winter wheat is not widely grown in the harsh winters of the extreme Northern Plains due to the higher risk of winter damage. However, the high yield potential of winter wheat verses spring wheat has driven the willingness of many producers to take the risk of planting it during the winter season (Wiersma et al., 2006). In contrast, producers in Deep South Texas typically do not grow winter wheat due to its vernalization requirements, which are prolonged exposure to low temperatures that will allow flowering to occur (Loukoianov et al., 2005). Winter wheat is at risk of not being vernalized in Texas south of College Station at latitude 29 degrees N, thus it is less likely flower, set seed or yield grain. Furthermore there is a risk that vernalization may not occur in Texas if the plants emerge late. It is difficult to know the exact number of days required for vernalization to take place on all winter wheat varieties (Morgan et al., 2006). With the higher yield potential of HRW, as opposed to HRS, many Texas producers look for choices when selecting a wheat variety for their region.

To extend the range of winter wheat cultivars that will perform well in South Texas, wheat breeders select for wheat varieties that are more photoperiod-insensitive and have shorter vernalization requirements. Some wheat cultivars require longer vernalization periods than others and can range from 5 to 45 days of accumulated exposure to temperature ranges of 32 to 45 ^{o} F. If the vernalization requirement is not met, winter wheat plants will remain in the vegetative state and will not produce grain

(Morgan et al., 2006). An increase in winter temperatures in South Texas could have a negative impact on winter wheat production due to incomplete vernalization.

Photoperiod lengths vary in the U.S. depending on the longitudinal location during the growing season. In the major growing regions of the U.S., from Central Texas to North Dakota, winter wheat is planted anywhere from September in the Great Plains to December in Central Texas. Wheat is generally classified as a long-day (LD) plant because, when exposed to longer days, it tends to flower earlier (Dubcovsky et al., 2006). A location such as Yoakum, TX (29.2911° North) never receives less than 10 hours of daylight from planting (December) until the March Equinox when all locations in the U.S. wheat growing areas receive 12 hours of day-length. In contrast, Wichita, KS (37.6889° North) receives less than 10 hours of day-length from planting (September) until the March Equinox. After the March Equinox when the day-length increases for all locations in the U.S. wheat growing area, stem elongation and head emergence begin to occur. In Yoakum, TX, stem elongation will begin in late March and finish up in April; whereas in Wichita, KS stem elongation will not occur until late April to May because of the shorter photoperiod lengths following planting. However with Yoakum receiving just over 13 hours of day light at the peak of heading and Wichita receiving just over 14 hours, wheat planted in that area of Kansas will rapidly complete its life cycle due to the longer days (Koning, 1994).

The goal of this project is to better understand the response of winter wheat to photoperiod and vernalization in order to successfully breed cultivars that are more adapted to South Texas where minimal vernalization requirements are necessary. In

this study, 48 HRW entries from the 2010 Southern Regional Performance Nursery (SRPN) were evaluated at 30 field locations across the U.S. Twenty of the 48 SRPN lines were selected to conduct additional growth chamber experiments.

The central hypothesis of this study is that better understanding of winter wheat response to photoperiod and vernalization will enable breeders to identify the genetic combinations necessary for adaptation across broad adaption zones. The specific objectives of this study were to: 1) Characterize U.S. Great Plains winter wheat genotypes for their vernalization and photoperiod response under growth chamber conditions; and 2) Associate main effects of photoperiod and vernalization with adaptation traits such as flowering dates, plant height, grain yield and its stability, in addition to vernalization and photoperiod marker data.

CHAPTER II

REVIEW OF LITERATURE

The development phases of winter wheat

There are ten major growth stages that wheat must go through to complete its life cycle. These are: germination, seedling, tillering, stem elongation, booting, heading, flowering, milk, dough and ripening (Fowler, 2002). Thermal time, the time/temperature relationship that controls growth rate and development in wheat, is measured in heat units, determined by averaging the minimum and maximum daily temperatures.

Winter wheat would typically need 2200 heat units to reach physiological maturity. For example, a minimum daily temperature of 10° C and maximum temperature of 20° C would earn 15 heat units where a winter wheat crop would be produced in 147 days, with 147x15=2205 heat units (Fowler, 2002). In contrast days at 0° C will have 0 heat units. . Ritchie et al. (1998) reported that the amount of light the plant receives over the optimal temperature range has a strong influence on the rate of biomass accumulation. The duration of growth is very much dependent on its thermal environment and also the photoperiod rate during floral induction. In modern annual cultivars variations occur more often in the duration of growth as opposed to the rate of growth. Older cultivars with the same duration of growth as modern cultivars tend to favor vegetative growth over the reproductive growth, which consequently lowers their harvest index (Ritchie et al, 1998).

Wheat development can be summarized into the three most important stages of its life cycle: stem elongation, heading, and physiological maturity. An important yield component aspect of development is the duration of time between any two of the stages (Chen et al., 2010). Chen et al., (2010) characterized these three most important developmental stages utilizing 350 markers that were mapped from a population of recombinant inbred lines (RILs) derived from a cross between 'Jagger' and '2174', both HRW cultivars. Control of variation in the development process was traced back to three major QTLs closely associated with known flowering genes, *VRN-A1, PPD-D1* and *VRN-D3*. Their findings suggested that one could regulate the various developmental phases to obtain the desired agronomic need by combination of the appropriate alleles at the three loci.

To better understand the genetics of vernalization defined by the promotion of flowering by cold treatment, they cloned a gene, *TaVRT-1* (*Triticum aestivum* vegetative to reproductive transition-1; Danyluk et al, 2003). This gene has been localized to the *Vrn-1* region that is associated with vernalization and tolerance to freezing temperatures. They discovered that the *TaVRT-1* developmental gene is integral in the regulatory pathways of transition from the vegetative to the reproductive phase in wheat. This transition is triggered by environmental cues such as photoperiod and vernalization and it's used by many plant species to regulate flowering during the year, and this has a critical impact on yield (Turner et al., 2013). Fowler and Limin (2007) noted that exposure of plants to temperatures that approach freezing turns on the LT tolerance genes. They also reported that the down regulation of the LT-tolerance genes is initiated

by the transition of plants from the vegetative to reproductive development phase. During the vegetative phase the cold-hardiness genes are fully expressed but once the plants enter the reproductive phase they have a limited ability to tolerate freezing temperatures (Fowler and Limin, 2007).

Vernalization response in winter wheat

Russian geneticist Trofim Lysenko, who studied the effects of cold temperatures on flowering, coined the term jarovization, which was translated from its original Russian to the English term vernalization. The first work cited on vernalization requirements of a wide range of plants was done by Gassner (1918). There are variations of vernalization requirements within a given species such as wheat.

HRW requires vernalization whereas spring wheat does not (Amasino, 2004). In winter wheat vernalization is mostly controlled by three *VRN1* loci**,** *VRN-A1, VRN-B1* and *VRN-D1*. Also noted were two other important genes *VRN2,* which is a long day flowering repressor of *VRN3* (Yan et al. 2003) Chen et al. (2009) reported that spring wheat carries the dominant VRN-*1* allele while winter wheat carries the recessive *vrn-1* allele (Chen et al., 2009). The majority of natural variation in vernalization response of winter wheat is controlled by the allelic differences in the MADS-box transcription factor of *VRN1*. *VRN1* expression is induced by the prolonged exposure to low temperatures during the winter vernalization period and also promotes the transition to the reproductive phase of the apical meristem (Chen and Dubcovsky, 2012). Chen et al. (2009) studied the association of VRN-A1 and stem elongation among 19 locally adapted winter wheat cultivars. These lines, which also included Jagger and 2174, were

genotyped using the VRN-1 marker data generated in their study. Chen et al. (2009) analyzed the data with previous first-hollow-stem (FHS) data, internode elongation that reaches approximately 1.5 cm by using thermal units. They found that 17 of the 19 cultivars in the study carried the same vrn-A1b allele as in 2174, which historically began stem elongation two weeks later than Jagger. They concluded that local breeders had inadvertently been selecting for this allele which contributed to later stem elongation and established development patterns.

Loukoianov et al. (2005) proposed a model illustratingVRN-1 being repressed by VRN-2 either directly or indirectly. During vernalization, VRN-2 is down-regulated and VRN-1 is released from its repression, which in turn initiates flowering, according to their model which is shown below (Figure 1).

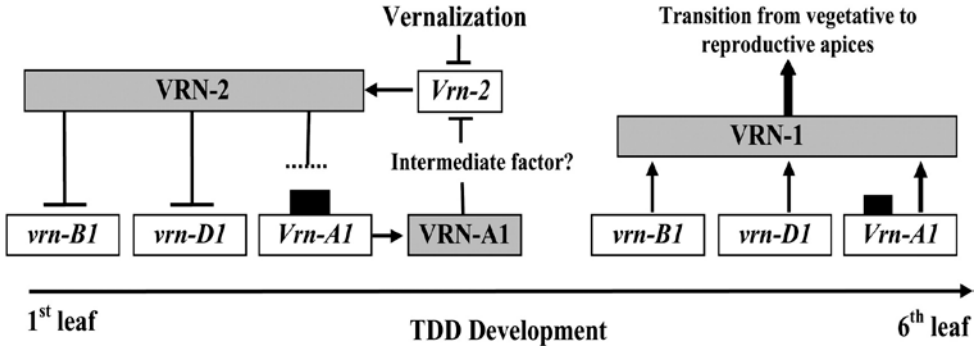

Fig. 1. Hypothetical model to explain the developmental progress in transcription initiation of dominant and recessive VRN-1 alleles in polyploid wheat TDD (Vrn-A1 vrn-B1 vrn-D1). Genes are in white boxes and proteins in gray boxes. Thin arrows indicate transcription/translation and "⊥" indicates repression. The black rectangle and dotted bar above the dominant Vrn-A1 allele indicate the lack of interaction with the VRN-2 repressor. According to this model, the initiation of transcription of the dominant Vrn-1 allele results in the direct or indirect down-regulation of VRN-2 transcription. As development progresses, the absence of the VRN-2 repressor allows transcription of the recessive vrn-1 alleles. Accumulation of the VRN-1 protein above a critical level triggers the transition to flowering (Loukoianov et al., 2005).

Dominant spring growth habit could be induced by a single VRN-1 allele not being repressed by VRN-2. Trevaskis et al. (2003) identified 10 wheat MADS box genes that were associated with the vegetative tissue and were expressed before floral transition. They discovered two vernalization-responsive MADS box genes with one of them being the hexaploid wheat orthologue of TMAP1 described as WAP1. WAP1 was identified as being the possible candidate for the VRN1 gene and has shown a strong expression in spring wheat but was not expressed in winter wheat until after vernalization was complete.

Diallo et al. (2012) looked at the possibility that the winter wheat genes TaVRN1, TaVRN2 and TaFT1 expression is in any way associated with the chromatin methylation state during periods of vernalization in wheat. They measured the level of the activator histone H3 trimethylation of lysine 4 (H3K4me3) and the repressor histone H3 trimethylation of lysine 27 (H3K27me3) at the promoter regions. Their study showed that histone methylation at the promoter level of TaVRN1 and TaFT1 were associated with the flowering transition in wheat. However these markers had less of an effect on the TaVRN2 repressor. They concluded that this may be associated with the cellular memory of vernalization in wheat (Diallo et al 2012).

Genetic mapping work done by Yan et al., (2006) showed complete linkage between VRN3 and Arabidopsis flowering locus T (FT), the signal that promotes flowering. They showed that the transcription levels of wheat orthologue TaFT are higher in homozygous dominant VRN3 alleles (early flowering) than that of plants homozygous

recessive vrn3 alleles (late flowering). Their results reinforce the idea that the FT genes of wheat and barley are the reason for their natural allelic variation and can be observed in the vernalization requirement of these crops, which in turn will provide additional sources of adaptive diversity (Yan et al., 2006).

Photoperiod responses in winter wheat

Photoperiodism is defined as the plants response to lengths of light and dark events within a 24 hour period. Plants are classified depending on their response to day-length. Plants can be categorized as either long-day (LDP) or short-day (SDP) if they are sensitive to photoperiods. There are also day-neutral plants (DNP), which are not sensitive to photoperiod changes. Within the categories of LDP and SDP, there are sub-categories of photoperiod types: qualitative (obligate), photoperiod being an absolute requirement and quantitative (facultative), which flower under either long day or short day conditions. Wheat would be considered as quantitative long-day plant because it flowers under short day conditions but long-day conditions will accelerate its flowering (Hopkins and Huner, 1995)

There are two types of photoperiods, photoperiod sensitive (PS) and photoperiod insensitive (PI). PS plants require long days before flowering will occur, whereas PI plants can flower in both short day and long day environments (Dyck et al.,2004). According to Dyck et al., (2004), plant breeders in the northern latitudes pay particular attention to photoperiod responses because PI cultivars have been shown to grow faster, have less frost damage and have greater yield potential.

In a photoperiod- by-temperature interaction study conducted by Slafer and Rawson (1995), the effects of varying photoperiod day length and various temperature ranges were reflected in the heading time in wheat. Two spring wheat cultivars, 'Sunset' and 'Condor', a semi winter wheat, 'Rosella', and a winter wheat, 'Cappelle Desprez', were evaluated for their response to photoperiod regimes of 9, 12, 15, 17, 19, and 21 hours and maximum-minimum temperature ranges between 21/17 and 16/12° C. They discovered that an increase in temperature and photoperiods always reduced time to heading although the degree of response to photoperiod was dependent on temperature and varied among genotypes. The winter wheat in their study had a qualitative response to photoperiod and did not head at all when exposed to photoperiods shorter than 12 hours. The spring wheat cultivar Sunset had a completely opposite reaction and showed a clear quantitative response with a steady delay in heading as photoperiod times decreased. Finally, the remaining spring wheat, Condor, and the semi-winter wheat Rosella had an intermediate quantitative response and achieved heading even under the shortest photoperiods, but had dramatic response under very short photoperiods. They concluded that for a particular genotype there is more than one degree of sensitivity to photoperiod response.

Worland et al, (1998) reported their 10 year evaluation of European wheat varieties and noted in their experiment that *Ppd1*, a PI allele, accelerated wheat ear emergence, reduce plant height, tillering and spike number. However, the allele caused an increase in spike fertility, resulting in increased grains per ear. They also noted the effects of *Ppd2*, a weaker gene for PI which exerts less significant pleiotropic effects

than *Ppd,* had a yield increase of 6% over *Ppd1*. They concluded that PI genotypes work best in southern European environments, whereas PS genotypes had more success in the northern European environments. Using 46 single chromosome recombinant lines (SCRLs), Khlestkina et al. (2009) studied a novel photoperiod response gene called *Ppd-B2*. They discovered upon mapping this gene that it was detected when plants were exposed to a long photoperiod, as opposed to *Ppd-1* which required short days for expression. The results of this study showed a link between *Ppd-B2* and an increase in grain protein content.

Bentley et al. (2011) screened a total of 1644 tetraploid and hexaploid wheat accessions for the *Ppd-A1a* allele. They showed that *Ppd-A1a* alleles were absent from wild tetraploid wheat and traditional hexaploid wheat but were predominate in modern durum wheat, suggesting that during durum cultivation they were selected for adaptation. CIMMYT developed some synthetic hexaploid wheat lines, by hybridizing elite durum wheat lines with *Aegilops tauschii* accessions in order to increase genetic diversity. The results showed that 71.4% of the 447 synthetic hexaploid and 9.6% of the 115 advanced lines carried the *Ppd-A1a* alleles from durum wheat. Upon backcrossing to hexaploid wheat, they showed that the durum *Ppd-A1a* alleles conferred a PI phenotype, which could give new sources of variation in flowering time in hexaploid wheat. Klaimi and Qualset (1973) study involved the inheritance of photoperiodic response of crosses made between four spring and three winter wheat lines. The seven parental lines where then separated into groups based on their photoperiod sensitivity. They noted that alleles present in the parents would dictate the photoperiod response of

the progeny and that daylength insensitivity was not always dominant to daylength sensitive cultivars. It was concluded that both additive and dominance factors as well as epistasis play a major role in regulation of photoperiod sensitivity.

Photoperiod and vernalization interaction of winter wheat

Temperate cereals usually are categorized by their response to extended periods of cold (vernalization) and daylength requirements (photoperiods). Danyluk et al. (2003) described the two developmental controlling features, vernalization requirement, which delays heading by postponing the transition from vegetative to reproductive phase and photoperiod requirement, which will only allow flowering to occur when optimal inducing conditions exist.

Wheat that is planted in the autumn has a vernalization requirement that will promote flowering and generally has an accelerated flowering response under LD conditions. However, wheat that is spring-planted does not have a vernalization requirement and can have a weak or strong response to LD conditions (Cockram et al, 2007).

The wide range of environments that wheat is adapted to could be linked to the allelic diversity in the genes regulating growth habit (VRN genes) and photoperiod responses (PPD genes). The differences in VRN genes categorizes wheat as either a spring or winter type, whereas the differences in the PPD genes would characterize the wheat as being either photoperiod-sensitive or photoperiod-insensitive (Distelfeld and Dubcovsky, 2009). A complex interaction of temperature and photoperiod is needed in order for flowering of wheat to occur (Masle et al., 1989).

In a study conducted by Dubcovsky et al. (2005), the effect of photoperiod on the regulation of wheat vernalization genes were examined. They noted by interrupting the LD (long day) treatment by 6 weeks of SD (short day) in some genotypes they were able to replace the vernalization requirement. This result was attributed to the SD down regulation of VRN2 flowering repressor, and was observed in photoperiod sensitive wheat. They concluded that the SD, down regulation of VRN2 repressor is probably a part of the SD-LD mechanism associated with the flowering of photoperiod sensitive winter wheat.

The phenomenon of "short day vernalization" was also described in Turner et al. (2013) as being the ability of some winterwheat varieties ability to flower after a period of growth in SD conditions under non vernalizing temperatures (Purvis and Gregory, 1937; Roberts et al.1988; Turner, 2013). It was noted that varieties that failed to flower under SD vernalization conditions were most often known to carry the photoperiod-insensitive (day neutral) *Ppd1* mutation. They concluded that photoperiod-sensitive winter wheat varieties will flower under SD conditions no matter the vernalization treatment because *Vrn-2* is not expressed in SD's and there is no photoperiod promotion.

Gonzalez et al., (2002) studied the effects of vernalization and photoperiod responses in the pre-flowering reproductive phase of wheat in the field. They sampled three high yielding wheat cultivars and subjected them to two vernalization regimes (non-vernalized and vernalized for 56 days) and four photoperiod regimes (natural photoperiod-NP, NP+2, NP+4 and NP+6),to study the effects of the different combinations of vernalization and photoperiod on the pre-flowering development of

wheat. They noted that when cultivars with a typically strong vernalization response did not meet their requirements, the duration of pre-flowering reproduction increased and spikelet initiation decreased. They concluded that, depending on the level of vernalization satisfaction received, the length of the vegetative and late pre-flowering reproductive phases changes.

Davidson et al., (1985) conducted an experiment to evaluate the effects of vernalization and photoperiod on heading date of 68 Australian and 49 exotic wheat lines. Their experiment examined the effects of the following treatments: no vernalization- natural photoperiod, 6 week vernalization-natural photoperiod, no vernalization- 16 hour photoperiod, and 6 week vernalization- 16 hour photoperiod. Their results indicated that all varieties in this study flowered and the time to ear emergence was greatest under the no vernalization and natural photoperiod, but was reduced by long photoperiods or vernalization, and at times the interaction of the two. They also noted that in spring wheat varieties ear emergence was increased 9 days by vernalization treatments and 43 days by long photoperiods; whereas in winter wheat varieties, ear emergence increased by 38 days due to vernalization treatments and 26 days under long photoperiods. The photoperiod response was dominated in 74 of the spring wheat lines and only four of the winter wheat varieties; however, vernalization had major influence in 22 of the winter wheat varieties and photoperiod in only 10. Under natural photoperiods, advancing ear emergence by 40 or more days in only 4 varieties indicates that the response to vernalization was relatively small. In contrast, vernalization effects were much more pronounced under long photoperiods.

In a study of photoperiod and vernalization response of wheat under controlled environment, Ortiz-Ferrara et al., (1995), noted that an increase of photoperiod and vernalization would result in early flowering. They looked at the effects of vernalized and non-vernalized plants under three photoperiod regimes (8, 12, 16 hours of daylength) on 20 wheat genotypes. They also found that the effect of vernalization was more pronounced under a short day cycle of 8 hours. The goal of the study was to evaluate two different screening techniques for wheat genotypes and their response to vernalization and photoperiod treatments. They utilized a growth chamber and greenhouse as their controlled environment which enabled them to compare their results with field screening techniques. They concluded that screening for vernalization response could be conducted in the field where large numbers of lines can be screened. Additional screening can be carried out on selected lines for daylength sensitivity, vernalization requirements and their interaction under greenhouse conditions using a 12 and 16 hour daylength.

CHAPTER III

EVALUATION OF WINTER WHEAT GENOTYPES IN A FIELD AND CONTROLLED ENVIRONMENT FOR THEIR RESPONSE TO PHOTOPERIOD AND VERNALIZATION

Introduction

Winter wheat production in Texas varies from year to year, predominantly depending on environmental conditions during the growing season. There was a 40% drop in yield from 2012 to 2013 due to severe drought and multiple freeze events in the Texas High Plains and Rolling Plains, according to Texas A&M AgriLife Research and Extension (Neely et al. 2013). More broadly adapted cultivars would open up areas of Texas that may not currently have high wheat production.

The majority of winter wheat production in Texas has been limited to the High Plains, Rolling Plains, and Northeastern parts, due to colder environments that would allow vernalization to take place. In the U.S. Southern Great Plains hard red winter wheat (HRW) requires a vernalization period of 2-6 weeks at 2-8° C, which is usually met above the XX latitude, except when the winters are warmer than average (Wang et al., 2009).

The main objective of this study was to characterize U.S. Great Plains HRW genotypes for their vernalization and photoperiod response, and to understand the association of this response with adaptation traits and marker data generated by the USDA-ARS Genotyping Laboratory in Manhattan, KS.

Materials and methods

Plant material

The Southern Regional Performance Nursery (SRPN) is a HRW Regional Nursery that is coordinated annually by the USDA-ARS at Lincoln, NE. The 2010 SRPN was planted across 30 field locations in eight U.S. states (Table 1). The 48 entries of the 2010 SRPN lines are listed below (Table 2). Twenty out of the 48 2010 SRPN experimental lines were selected for evaluation under growth chamber conditions in 2010, 2011, and 2012 (Table 3). The 20 lines, selected based on region of origin and agronomic traits, included four checks, namely 'Kharkof'(Graybosch and Perterson, 2010), 'Scout 66'(Cltr 13996), 'TAM107' (PI495594) and 'Fuller' (PVP 200800130).

Table 1. 2010 Southern Regional Performance Nursery (SRPN) locations.

State	Locations	State	Locations
Colorado	Akron	Oklahoma	Goodwell
	Burlington		Granite
	Ft. Collins Irrigated		Lahoma
	Walsh		Stillwater
Kansas	Colby	Texas	Bushland Dryland
	Garden City		Bushland Irrigated
	Hays		Chilicothe
	Hutchinson		Prosper
	Salina		
	Wichita		
	Winfield		
Nebraska	Alliance	South Dakota	Brookings
	Clay Center		Dakota Lakes
	Lincoln		
	North Platte		
	Sidney		
New Mexico	Clovis Dryland	Wyoming	Pine Bluffs
	Clovis Irrigated		
	Farmington Irrigated		

Table 2. 2010 Southern Regional Performance Nursery (SRPN) list of entries.

Entry	Line	Class	Pedigree	Program Source
1	Kharkof	HRW	Kharkof	check
2	Scout 66	HRW	Scout 66	check
3	TAM-107	HRW	TAM-107	check
4	Fuller	HRW	Fuller	check
5	KS07HW52-5	HWW	KS025580(TREGO/CO960293)/KS02HW25(TGO/JGR 8W)	KSU-HAYS
6	KS08HW176-4	HWW	TREGO/JGR 8W	KSU-HAYS
7	OK05526	HRW	KS94U275/OK94P549 F4:12	OSU
8	OK05212	HRW	OK95616-1/Hickok//Betty F4:12	OSU
9	OK05204	HRW	SWM866442/OK95548 F4:12	OSU
10	OK05511	HRW	TAM 110/2174 F4:12	OSU
11	OK07231	HRW	OK92P577-(RMH 3099)/OK93P656-(RMH 3299) F4:10	OSU
12	T150-1	HRW	T81/T201	Trio
13	T166	HRW	T81/KS93U206	Trio
14	T168	HRW	T136/T151	Trio
15	T167	HRW	T81/T137	Trio
16	NE06545	HRW	KS92-946-B-15-1=(ABI86*3414/JAG//K92)/ALLIANCE	UNL
17	NE07444	HRW	KS96HW10-3=(KS91HW29// RIO BLANCO/KS91H184)/WAHOO/NE99585	UNL
18	NI07703	HRW	R-148 (G97343) =(919021/B725//K92)/NI00436 =(WI89-273-13/NE93427 (=BEZ 1/CTK78//ARTHUR/CTK78/3/BENNET/4/NORKAN)	UNL
19	NI08708	HRW	CO980829 (=Yuma/T-57//CO850034/3/4*Yuma/4/NEWS1)/Wesley	UNL
20	BC01007-7	HRW	W99-331/97x0906-8	AGRIPRO NORTH
21	BC01131-24	HRW	W99-429-1/W98-422	AGRIPRO NORTH
22	BC01139-1	HRW	W99-188$-1/BC950285G-1-2	AGRIPRO NORTH
23	00X0100-51	HRW	W95-301/W98-151	AGRIPRO NORTH

Table 2. Continued.

Entry	Line	Class	Pedigree	Program Source
24	HV9W06-1046	HRW	M97-1171/G980039//G982238	WestBred Haven
25	HV9W06-509	HRW	G982231/G982159//KS920709W	WestBred Haven
26	HV9W06-262	HRW	TX98U8134/3/KARL 92*2/RAVI-36	WestBred Haven
27	HV9W04-1594R	HRW	KS89180B-2-1-1/CMBW91M02959T//JGR	WestBred Haven
28	CO04393	HRW	Stanton/CO950043	CSU
29	CO04499	HRW	Above/Stanton	CSU
30	CO050270	HRW	Hatcher/NW97S295	CSU
31	CO050303-2	HRW	CO980829/TAM 111	CSU
32	CO050322	HRW	CO980829/TAM 111	CSU
33	CO050337-2	HRW	CO980829/TAM 111	CSU
34	KS010990M~8	HRW	Trego/Ventnor//KS940786-6-4	KSU-Manhattan
35	KS06O3A~50-3	HRW	OVERLEY*3/AMADINA	KSU-Manhattan
36	KS06O3A~58-2	HRW	OVERLEY*3/AMADINA	KSU-Manhattan
37	KS011327M~2	HRW	KS940748-2-4/TX97V4311//Overley	KSU-Manhattan
38	OK07209	HRW	OK93P656-(RMH 3299)/OK99621 F4:10	OSU
39	TX05A001822	HRW	2145/X940786-6-7	TAMU
40	TX06A001263	HRW	TX97V3006/TX98V6239	TAMU
41	TX06A001132	HRW	HBG0358/4/T107//TX78V3620/Ctk78/3/TX87V1233	TAMU
42	TX06A001281	HRW	TX98VR8422/U3704A-7-7	TAMU
43	TX06A001386	HRW	TX99A6030/CUSTER	TAMU
44	TX05V7259	HRW	T107//TX78V3620/Ctk78/3/TX87V1233/4/Arap//TX86V1540/T200	TAMU
45	TX05V7269	HRW	HBG0358/4/T107//TX78V3620/Ctk78/3/TX87V1233	TAMU
46	TX05A001188	HRW	T107//TX98V3620/Ctk78/3/TX87V1233/4/N87V106//TX86V1540/T200	TAMU
47	BC01138-5	HRW	W99-188$/BC950814-1-1	AGRIPRO South
48	AP06T3621	HRW	X920232-5/Karl 92//X920750A-13-1	AGRIPRO South

Entry number and pedigrees according to USDA-ARS 2010 Southern Regional Performance nursery Graybosh (2010). HRW-hard red winter wheat, Programs from which source seed was provided.

Table 3.The 2010 Southern Regional Performance Nursery (SRPN) growth chamber entries.

Entry	Line	**Class**	Pedigree	Program Source
1	Kharkof	HRW	Kharkof	check
2	Scout 66	HRW	Scout 66	check
3	TAM-107	HRW	TAM-107	check
4	Fuller	HRW	Fuller	check
5	KS07HW52-5	HWW	KS025580(TREGO/CO960293)/KS02HW25(TGO/JGR 8W)	KSU-HAYS
6	OK05526	HRW	KS94U275/OK94P549 F4:12	OSU
7	OK05204	HRW	SWM866442/OK95548 F4:12	OSU
8	OK07231	HRW	OK92P577-(RMH 3099)/OK93P656-(RMH 3299) F4:10	OSU
9	NE07444	HRW	KS96HW10-3=(KS91HW29// RIO BLANCO/KS91H184)/WAHOO/NE99585	UNL
10	NI07703	HRW	R-148 (G97343) =(919021/B725//K92)/NI00436 =(WI89-273-13/NE93427 (=BEZ 1/CTK78//ARTHUR/CTK78/3/BENNET/4/NORKAN)	UNL
11	NI08708	HRW	CO980829 (=Yuma/T-57//CO850034/3/4*Yuma/4/NEWS1)/Wesley	UNL
12	CO050270	HRW	Hatcher/NW97S295	CSU
13	CO050303-2	HRW	CO980829/TAM 111	CSU
14	KS010990M~8	HRW	Trego/Ventnor//KS940786-6-4	KSU-Manhattan
15	KS06O3A~50-3	HRW	OVERLEY*3/AMADINA	KSU-Manhattan
16	KS011327M~2	HRW	KS940748-2-4/TX97V4311//Overley	KSU-Manhattan
17	TX06A001281	HRW	TX98VR8422/U3704A-7-7	TAMU
18	TX06A001386	HRW	TX99A6030/CUSTER	TAMU
19	TX05V7259	HRW	T107//TX78V3620/Ctk78/3/TX87V1233/4/Arap//TX86V1540/T200	TAMU
20	TX05V7269	HRW	HBG0358/4/T107//TX78V3620/Ctk78/3/TX87V1233	TAMU

Entry number used in growth chamber experiment, not the same entry number as original 2010 Southern Regional Performance list. Pedigrees according to USDA-ARS 2010 Southern Regional Performance nursery Graybosh (2010). HRW-hard red winter wheat, Programs for which source seed was provide.

Experimental design

Three growth chamber cycles were conducted in this study. In the first (2010) and second (2011) growth chamber cycle, seeds from the selected lines were vernalized in petri dishes by germinating twenty seeds at room temperature for 30 hr, then continuing the growing cycle in a refrigerator at 1-2° C. The sprouted seeds were left under dark refrigeration for six weeks according to Davidson et al. (1985). Three seeds were planted per six-inch, pot with two pots per variety into a soil medium. Each pot per variety represented a replication. A parallel set was planted without exposure to vernalizing temperatures to represent the non-vernalized set according to Darapuneni et al., 2013. Both sets of pots were then placed in a split-split plot arrangement in two growth chambers and rotated weekly to equalize temperature and light fluctuation within the chamber. The chambers were set for 12 and 16 hour day-lengths. Data was taken on flowering and heading dates of all lines. The same experiment was repeated in 2011. The 2012 growth chamber experiment included two vernalization treatments (3 and 6 weeks) and two photoperiod lengths (10hr and 14hr).

Data collection

Flowering dates were recorded when 50% of the tillers in the pot showed visible anther extrusion and or when trapped anthers changed from green to yellow. Data compiled by the USDA-ARS on the remaining 30 field locations were utilized including; grain yield, heading date, yield stability and height. Genotyping was conducted at the USDA-ARS genotyping lab in Manhattan, KS, using KASP chemistry in a K-Biosciences SNP pipeline to assess marker data of the lines used in this experiment. The

markers considered for this experiment were photoperiod marker PPD-D1 LD and markers for the three vernalization genes *Vrn-A1, Vrn-A1b* and *Vrn-D3.*

Basal Vegetative period (BVP), i.e. intrinsic earliness, was measured as time for vernalized seedling (6 week) to grow to anthesis in the longer photoperiod (16 hour), based on testing conducted in the 2010 and 2011 growth chamber evaluations. Furthermore, the main effect of vernalization (ΔV) was measured as the difference in anthesis date between vernalized (6 wk) and moderately vernalized (3 wk) seedlings under medium long day (14 h) conditions, based on testing conducted in the 2012 growth chamber evaluation. The main effects of photoperiod (ΔP) were measured as difference in days to anthesis between 16 and 12 h treatments in the vernalized seedlings (6 wk vernalization) measurements from growth chamber experiment evaluations done in 2011 and 2012.

Statistical analysis

Growth chamber data were analyzed as a split-split plot design using SAS 9.3. The ANOVA tables of the 2010 and 2011 growth chamber experiments showed the main plot being photoperiod (16 hour vs. 12 hour), the sub-plot being vernalization treatment (6 week vs. 0 week), and the sub-sub plot being genotype (Table 4). Furthermore, the ANOVA table of the 2012 growth chamber experiment shows the main plot representing photoperiod (14 hour vs. 10 hour), the sub-plot representing vernalization treatment (6 week vs. 3 week), and the sub-sub plot represented by genotype, and can be seen on the second table on page 28.

Biplot analysis

The GGEbiplot software of Yan and Kang (2003), was used to generate the biplots illustrated in the results section . A two-way matrix of genotypes as entries and traits as testers was generated from mean values for genotypes. Rows and columns were treated as entries and testers, respectively. The biplot model was as follows:

$$Y_{ij} - \mu - \beta_j = \lambda_1 \xi_{i1} \eta_{j1} + \lambda_2 \xi_{i2} \eta_{j2} + \in_{ij}$$

where = Y_{ij} expected value of entry i and tester j, μ = grand mean, β_j = mean of all crosses to j, λ_1= PC1, ξ_{i1}= PC1 eigenvector of entry i, η_{j1}= PC1 eigenvector of tester j, λ_2= PC2, ξ_{i2}= PC2 eigenvector of entry i, η_{j2}= PC2 eigenvector of tester j, and $\in_{ij}$= residual of model associated with combinations of entry i and tester j.

According to Yan and Tinker (2006) traits with acute angles are positively associated while obtuse angles indicated a negative association. Traits with near right angles are independent. Entries close to one another signify similar trait profiles and entries opposite one another relative to the origin signify opposite trait profiles. Performance of an entry with regard to a trait is better than average if the angle between its vector and the trait's vector is less than 90°; lower than average if greater than 90°; and near average if the angle is near 90°.

For genotype-by-environment or genotype-by-trait interactions, it is necessary to establish whether or not there are relevant rank changes of a specific genotype across a given environment or for a given trait. For example, HRW is expected to have longer days to heading in the northern Great Plains as opposed to South Texas. If there are not relevant rank changes, a particular line(s) may be identified as early across all environments. A biplot allows a breeder to determine whether or not a single environment should be divided into multiple mega-environments to exploit or avoid any potential genotype-by-environment interactions. A biplot can also assist a breeder in identifying the sources of these interactions. The most ideal test environments and superior genotypes can be identified through the use of biplot analysis (Yan and Tinker, 2006).

Two types of biplot views were generated for analysis of the HRW set in this study. These views include a mean performance and stability view of genotypes and a which-won-where view.

Table 4. Split-split plot analysis of 2010 and 2011data with the main plot being photoperiod (16 vs. 12 hour), the sub-plot being vernalization (6 vs. 0 week), and the sub-sub plot being genotype.

Source	DF	SS	MS	F value	P value
Year	1	2514.4031	2514.4031**	32184.4	0.0035
Error a = rep(year)	2	0.0813	0.0406	0.00	0.9966
P	1	7097.0281	7097.0281*	3115.29	0.0114
year*P	1	100.1281	100.1281NS	43.95	0.0953
Error b = rep*P	1	2.2781	2.2781	0.19	0.6637
V	1	317079.1531	317079.1531**	277987	<.0001
year*V	1	2514.4031	2514.4031**	2204.41	0.0005
P*V	1	7097.0281	7097.0281**	6222.05	0.0002
year*P*V	1	100.1281	100.1281*	87.78	0.0112
Error c = rep*P*V	2	2.2813	1.1406	0.10	0.9094
Genotype	19	5900.2844	310.5413**	25.87	<.0001
P*Genotype	19	1525.9094	80.3110**	6.69	<.0001
V*Genotype	19	5900.2844	310.5413**	25.87	<.0001
P*V*Genotype	19	1525.9094	80.3110**	6.69	<.0001
year*Genotype	19	1609.0344	84.6860**	7.05	<.0001
year*P*V*Genotype	57	2872.6531	50.3974**	4.20	<.0001
Error d = residual	155	1860.8594	12.0055		

Coefficient of variation (CV%) = 11.00
NS, *, and ** = Significant at 5% and 1% respectively; P=photoperiod, V=vernalization

Table 5. Main effects and interaction of photoperiod and vernalization for days to anthesis of 20 hard red winter wheat lines tested in the growth chamber in 2010 and 2011.

	2010			2011		
Photoperiod (h)	**HV**	**NV**	**Mean**	**HV**	**NV**	**Mean**
12	**65.65**	**0.00**	**32.83**	**79.1**	**0.0**	**39.6**
16	**49.05**	**0.00**	**24.53**	**58.0**	**0.0**	**29.0**
Mean	**57.35**	**0.00**	**28.7**	**68.6**	**0.0**	**34.3**
CV%			**7.9%**			**12.8%**
LSD 0.05 (P)			**0.95**			**5.24**
LSD 0.05 (V)			**0.24**			**1.26**

Photoperiod 12 hr and 16 hr, HV 6 weeks of vernalization, NV 0 weeks of vernalization.

Table 6. Split-split plot analysis of 2012 data with the main plot being photoperiod (14 vs. 10 hour), the sub-plot representing vernalization (6 vs. 3 week), and the sub-sub plot representing genotype.

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Rep	1	212.7198	212.7198	0.24	0.6269
P	1	303055.1555	303055.1555	6.68E7	<.0001
Error a = REP*P	1	0.0002	0.0002	0.00	0.9996
V	1	4305.0156	4305.0156	12.73	0.0703
P*V	1	34727.0582	34727.0582	102.40	0.0096
Error b = REP*P*V	2	804.0872	402.0436	0.45	0.6391
Name	18	27367.9625	1520.4424	1.70	0.0588
P*Name	18	38718.9917	2151.0551	2.41	0.0046
V*Name	18	26166.2202	1453.6789	1.63	0.0758
P*V*Name	18	21431.5903	1190.6439	1.33	0.1940
Error c = residual	71	63360.9868	892.4083		
Corrected total	150	520149.7881			

CV% = 55.1

Table 7. Main effects and interaction of photoperiod and vernalization for days to anthesis of 20 hard red winter wheat lines tested in the growth chamber in 2012.

	2012		
Photoperiod (h)	**HV**	**MV**	**Mean**
10	**19.5**	**0.00**	**9.7**
14	**78.4**	**119.7**	**99.3**
Mean	**48.5**	**59.8**	**55.0**
CV%			**55.1**
LSD 0.05 (P)			**00.14**
LSD 0.05 (V)			**12.7**

Photoperiod 10 hr and 14 hr, HV 6 weeks of vernalization, MV 3 weeks of vernalization

Results and discussion

Analysis of variance

In the 2010-2011 growth chamber analysis all effects and first-order and second-order interactions were significant at the 5% level of probability. We had a significant photoperiod-by-vernalization-by-genotype interaction, indicating that genotypes responded differently across different day lengths and vernalization treatments (Table 4) Table 5 shows the main effects and interaction of photoperiod and vernalization for the 2010-2011 growth chamber experiments. Days to anthesis decreased significantly ($P < 0.005$) as day-length increased.

In the 2012 growth chamber analysis a significant photoperiod-by-vernalization interaction was observed, indicating that the averaged flowering date for all 20 genotypes varied significantly across different vernalization and photoperiod treatments (Table 6). Table 7 shows the main effects and interaction of photoperiod and vernalization, based on days to anthesis, for the 2012 growth chamber experiment. The results showed that fully vernalized (6 weeks) plants flowered earlier than partially

vernalized ones (3 weeks).This indicates that it will be difficult to predict line performance without testing under varying photoperiod and vernalization conditions, which concurs with Ortiz-Ferrara et al. (1995), in which they concluded that without simple screening of these traits, adaptation of wheat to broad growing regions would be limited.

A summary of the means of the lines for BVP, the main effects of photoperiod (ΔP) and vernalization (ΔV) from the growth chamber flowering date results, as well as the four markers associated with photoperiod and vernalization, plant height, days to heading, yield and yield stability across the 2010 SRPN locations are compiled in Table 8. The BVP of the genotypes varied greatly as they did in other studies (Ortiz-Ferrara et al., 1995) and ranged from 42.3 to 76.5 day in this study.

The ΔP, the difference in flowering dates from the longer day photoperiod at 16 hours verses the shorter day photoperiod at 12 hours ranged from 6.8 days to anthesis (DTA) to 43 DTA. There were eight lines that tested photoperiod insensitive, nine sensitive, two unknown and 1 heterogeneous for the PPD-D1 gene. The ΔP for lines that tested photoperiod insensitive based on PPD-1 ranged from 6.8 to 21 DTA. The ΔP for lines that tested photoperiod sensitive based on PPD-1 ranged from 10.3 to 43 DTA. As noted in Ortiz-Ferrara et al., 1995, the long day requirement in photoperiod sensitive wheat is not overcome by prolonged vernalization. The longest ΔP of 43 DTA was observed in old cultivar Scout66. The photoperiod insensitive wheat with the lowest DTA (6.8) was OK07231, which could confirm that the PPD-D1 insensitivity gene was expressed in this un-released advanced line. However, the genotype NI07703, which

tested PPD-D1 insensitive but with growth chamber measured ΔP of 21.3 could point to a false negative PPD-D1 data point or interaction between photoperiod sensitivity and vernalization requirement. This could confirm statements made by other researchers that a complex interaction between vernalization and photoperiod exists (Stefany, 1993). Not specific to the photoperiod sensitivity of a particular HRW cultivar, other studies have noted that with an increase in photoperiod length there is a decrease in the vegetative phase of wheat (Gonzalez et al., 2001).

The ΔV, the difference in flowering dates from the (6 wk) vernalization to the moderately vernalized (3 wk), under a medium long day (14hr), ranged from 0 to 100 DTA. There were three genes evaluated in this study; vrn-A1, vrn-A1b and vrn-D3. HRW can be classified into three distinct groups based on response to the low temperature requirement needed to reach vernalization saturation point. According to Li et al., (2013) a weak winter type would need less than 2 weeks, a semi winter type would need between 2-4 weeks and a strong winter type would require more than 4 weeks of vernalization. In this study there were 14, 3, and 3 lines that tested weak winter, intermediate, and heterogeneous, respectively based on the vrn-A1 gene. The ΔV values for lines that tested weak winter based on vrn-A1 ranged from 0 to 91 DTA. On the other hand, the ΔV values for lines that tested intermediate winter, based on vrn-A1 marker, ranged from 26 to 100 DTA. Furthermore, the ΔV values for the lines that tested heterogeneous winter based on vrn-A1 were 0 to 47.5 DTA. It is worth noting that Kharkof has a ΔV of 91 DTA but tested vrn-A1 weak winter, whereas TX06A1281 has a ΔV of 26 DTA and tested vrn-A1 intermediate winter. The discrepancy could be

explained by genotyping error, an interaction with other vernalization genes in this study, or of most interest, additional unreported genes that condition it's unique response.

The Vrn-A1 marker was used to detect HRW alleles associated with early stem elongation, and it is associated with late stem elongation, according to the USDA's 2010 SRPN marker data. Seventeen of the 20 lines evaluated in this study tested as having vrn-A1b for late stem elongation, and the reaming three tested as having vrn-A1a for early stem elongation. In this study the vrn-D3 gene with alleles vrn-D3a, which is associated with earlier maturing HRW, had 12 lines, and vrn-D3b, which is associated with late maturing HRW was positive in eight lines. The two lines with the highest ΔV were TX05V7259 (100 DTA) and Kharkof (91 DTA). Both lines were photoperiod sensitive and vrn-A1b positive for late stem elongation; however, Kharkof which, on average across all location was 8 days later in the field trials than TX05V7259, tested as a weak winter vrn-A1 and late winter based on vrn-D3. Noted in a study conducted by Chen et al., (2010) in winter wheat cultivar Jagger, Vrn-A1 and Vrn-D3 alleles accelerated phonological development and PPD-1 decreased it due to Jagger photoperiod sensitivity.

In this study it is believed that a certain combination of genes would dictate the acceptable length of time a variety needs to be vernalized and receives the proper photoperiod length in order to complete its life cycle. As concluded in the study done by Chen et al., (2010), the correct combinations of alleles at loci Vrn-A1, Vrn-D1 and Ppd-1 would regulate the developmental phases in wheat and in turn could be customized to

fit various agricultural needs. A clear distinction can be made between lines that perform well in Texas and those that have underperformed. Newer lines such as TX06A1281, which is PPD-D1 LD insensitive, VRN-A1 inter-winter and VRN-D3 early winter, seem to outperform older lines Such as Kharkof which tested PPD-D1 LD sensitive, VRN-A1 weak winter and VRN-D3 late winter. Countries in the northern latitudes such as France and the UK would typically grow photoperiod sensitive wheat Worland et al. (1998). In contrast, in those countries in the southern latitudes of Europe, such as Italy and Yugoslavia, photoperiod insensitive wheat cultivars are more commonly grown. However some inconsistencies have been noted of older varieties and landraces being grown in some southern European areas as more photoperiod sensitive than newer lines Worland et al. (1998). Wheat breeders in southern Europe have improved adaptability by producing photoperiod insensitive wheat.

Table 8 Main effects of photoperiod and vernalization.

Name	BVP	ΔV	ΔP	PPD-D1	Vrn-A1	Vrn-A1b	Vrn-D3	Height (cm)	Heading (day)	Yield	Stability
Fuller	55.5		11.5	Sensitive	WeakWinter	Late	EarlyWinter	80	137	3512	1.1
NI07703	47.3	0	21.3	Insensitive	HeteroWinter	Late	EarlyWinter	81	139	3665	1.1
OK07231	54.3	0	6.8	Insensitive	WeakWinter	Late	Latewinter	80	141	3912	1.0
OK05526	46.8	12	15	Insensitive	HeteroWinter	Late	EarlyWinter	84	138	3876	1.0
KS010990M_	52.5	16.5	23.5	Unknown	WeakWinter	Early	Latewinter	82	141	3379	0.9
KS07HW525	53.3	20.1	13.3	Sensitive	WeakWinter	Late	EarlyWinter	75	139	3480	1.2
CO050270	47.8	23.5	19	Insensitive	WeakWinter	Late	EarlyWinter	79	136	3739	1.3
KS011327M_2	68.3	23.5	10.3	Sensitive	WeakWinter	Early	EarlyWinter	84	139	3556	1.0
TX06A1281	42.3	26	20.8	Insensitive	InterWinter	Late	EarlyWinter	75	136	3670	1.0
TX06A1386	51.3	29	12.8	Insensitive	WeakWinter	Late	EarlyWinter	83	139	3687	1.0
KS06O3A_50	49.5	32.5	19.3	Unknown	WeakWinter	Early	Latewinter	84	137	3426	0.9
NE07444	47.8	46	9.5	Insensitive	WeakWinter	Late	Latewinter	86	139	3451	0.8
NI08708	60	47.5	13	Insensitive	WeakWinter	Late	EarlyWinter	80	140	3881	1.0
TX05V7269	56.3	47.5	14.8	Sensitive	HeteroWinter	Late	Latewinter	80	140	3877	1.2
TAM107	45.8	57	33.8	Sensitive	WeakWinter	Late	EarlyWinter	75	137	3047	0.9
CO050303_	76.5	63	15.8	Sensitive	InterWinter	Late	Latewinter	85	142	3908	1.1
OK05204	51.3	68	19.8	Hetero	WeakWinter	Late	Latewinter	82	141	3736	1.0
Scout66	51.3	76.5	43	Sensitive	WeakWinter	Late	EarlyWinter	94	141	2861	0.6
Kharkof	65.5	91	32	Sensitive	WeakWinter	Late	Latewinter	102	146	2323	0.5
TX05V7259	48	100	22	Sensitive	InterWinter	Late	EarlyWinter	78	138	3839	1.0

Correlation analysis

The results of Pearson's correlation coefficients among grain yield, yield stability, days to heading, plant height, BVP, ΔV, and ΔP (Table 9) illustrate that the taller HRW lines in this study were generally late maturing, poor yielding and less stable across environments. The photoperiod-sensitive lines also proved to be lower yielding and unstable across locations. This is not a coincidence as a strongly positive relationship between plant height and photoperiod sensitivity has previously been noted Borojevic and Borojevic (2005). Both Rht8 (reduced height gene) and Ppd-D1 (daylight insensitivity gene) are both on chromosome 2D, and together they have been shown to decrease flowering by eight days, gave a ten cm reduction in plant height and increased spikelet fertility. According to our study, lines that required longer vernalization times and were more sensitive to photoperiods were unstable across environments.

There was a positive correlation between photoperiod sensitivity and vernalization requirements ($r=0.55$, $P < 0.05$) as determined by our growth chamber evaluations. Static genotypes will perform well across environments and is dynamic genotypes if its performance continually changes with environmental changes Mohammadi and Amir (2013). Davidson et al., (1985) noted that time to heading was longest in non-vernalized plants under natural photoperiods, but was accelerated by long photoperiods. Plants exposed to natural photoperiods had smaller responses to vernalization. However, under longer photoperiods conditions, vernalization effects were much greater. Their study concluded that flowering of wheat is accelerated by long photoperiods and vernalization.

Table 9. Correlations among agronomic traits and the main effect of photoperiod (ΔP) and main effect of vernalization (ΔV).

	Heading	Yield	Stability	ΔP	ΔV	BVP
Height	0.73***	-0.66**	-0.77***	0.41^{ns}	0.43ns	0.41^{ns}
Heading		-0.42^{ns}	-0.51*	0.22^{ns}	0.43ns	0.62**
Yield			0.79***	-0.71**	-0.39ns	-0.06^{ns}
Stability				-0.55*	-0.46*	-0.03^{ns}
ΔP					0.55*	-0.18^{ns}
ΔV						0.21^{ns}

(-) indicates a negative correlation, *** high level of significance, **intermediate level of significance, * low level of significance, ns- not significant.

GGE biplot analysis

Genotype-by-trait biplot analysis provides a visual method for discerning the relationship among traits within a single or multitude of environments (Yang and Tinker, 2006). Furthermore, genotype-by-environment biplot analysis is a valuable visualization tool for evaluating multi-environment data, locations discriminating value and stability, and genotype-by-environment interaction. A biplot is a graphical display of a two-way table that breaks a product matrix into its column and row vectors (Yan and Tinker, 2006).

The "which-won-where" pattern of a GGE biplot dataset is considered one of its most attractive and illustrative views. In this view, a polygon is drawn on genotypes that are the farthest away from the biplot origin in order to encompass all other genotypes within the polygon's sides.

Genotypes located on the vertices of the polygon have the highest or lowest values for given traits in multiple environments (Yan and Tinker, 2006). Figures 2, 3, and 4 demonstrate which genotypes have the lowest or highest values for the main effect of photoperiod (ΔP), main effect for vernalization (ΔV), grain yield, height, and days to heading across the two growth chambers and all 30 field environments.

The Biplot for the relationships among photoperiod and vernalization explained 100% of total variation with 79.1% by PC1 and 20.9% by PC2 (Fig. 2). TX05V725 and Scout 66 were the vertex genotypes and the lines with the highest vernalization requirement and photoperiod sensitivity, respectfully. Kharkov was in the middle of the two, and had high ΔV and ΔP. On the other hand, genotypes Ok07231 and NI07703 had the lowest ΔV and ΔP, as they were the vertex genotypes located farthest from the ΔV and ΔP testers.

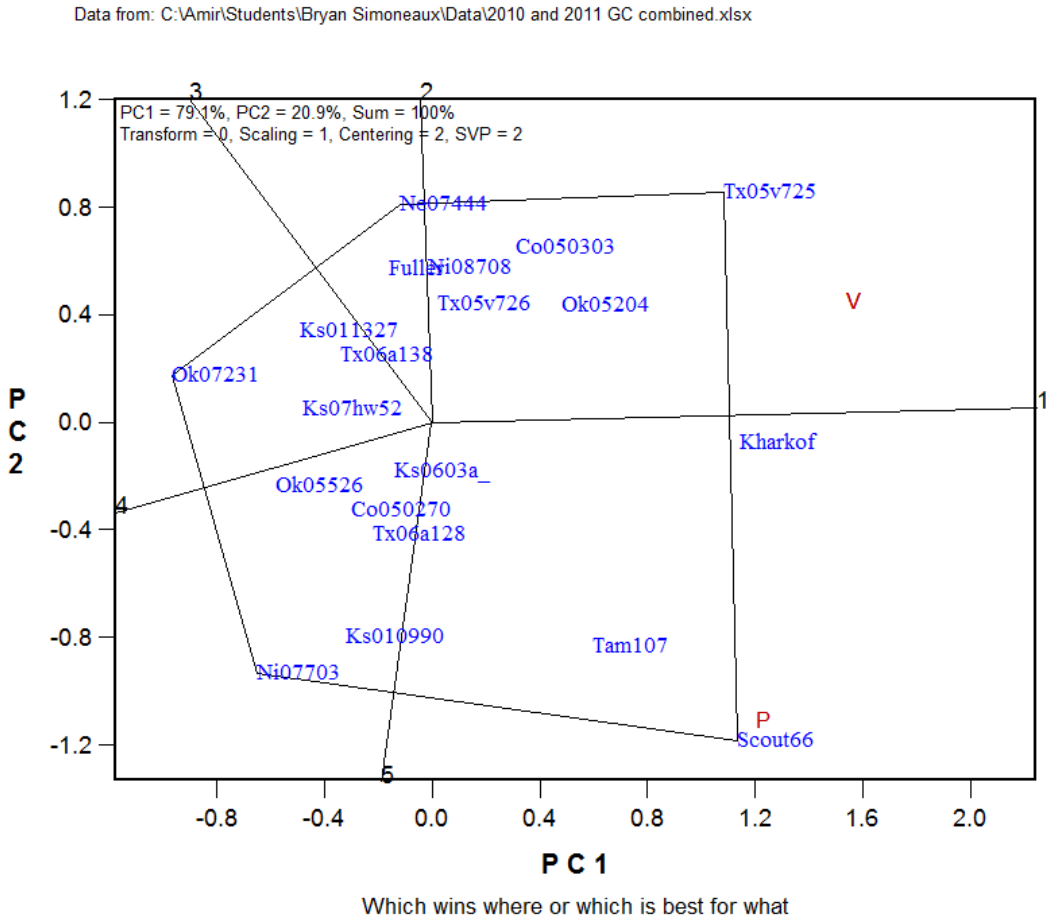

Fig. 2 Polygon view of biplot based on main effect of vernalization (V) and photoperiod (P) of 20 HRW lines tested in the growth chamber from 2010-2011. Genotypes that lie in the (V) quadrant have tested high for vernalization requirement and lines that lie in the (P) quadrant have tested to be photoperiod sensitive. Whereas lines that lie adjacent to the (P) quadrant have tested photoperiod in-sensitive. Lines that lie adjacent to the (V) quadrant have tested low in vernalization requirement.

The biplot for yield performance of the SRPN lines across all locations in the test (Table 10), explained 59.5% of the total variation with 49.9% by PC1 and 9.6% by PC2 (Fig.3). The biplot illustrates which lines performed well in terms of grain yield in which environments. TX05V7269 was the best overall genotype across all locations that fell within vectors 1 and 2. Genotypes TX06A1281, TX05V7259, KS07HW525 and CO050270 yielded higher in locations N1 and also performed well in locations C2, C3, K1, K2, K4, K5, N2, NE2, O1,O2, O4, T2, T4 and W1, which is illustrated by clusters 1 and 2 located between vectors 1 and 2. OK07231 was lower yielding than TXo5V7269 based on distance from the origin to the vertex of the biplot, but was the best entry for environments that fell within vectors 1 and 4. Genotype NI08708 did very well in location C4 and also along with NI07703, CO050303_2, and OK05204 did well in clusters 3, 4 and 5 located within vectors 1 and 4. These genotypes were closely associated with locations K3, NE1, NE4, S1, S2, T1, and T3 of vectors 1 and 4. Kharkov, Scout 66 and TAM 107 were the lowest yielders as they fell far from all location clusters.

Table 10. Locations, and their abbreviations, where the 2010 Southern Regional Performance Nursery (SRPN) was conducted.

Location	Abbreviations	Coordinates	Location	Abbreviations	Coordinates
Clovis Dryland	N1	34.4°N, 103.2°W	Hays	K1	38.8°N, 99.3°W
Clovis Irrigated	N2	34.4°N, 103.2°W	Hutchinson	K2	38.0°N, 97.9°W
Farmington	N3	36.7°N, 108.3°W	Salina	K3	38.8°N, 97.6°W
Bushland Dry	T1	35.1°N, 102.0°W	Colby	K4	39.3°N, 101.0°W
Bushland Irrigated	T2	35.1°N, 102.0°W	Garden City	K5	37.0°N, 100.0°W
Chilicothe	T3	34.2°N, 99.5°W	Wichita	K6	37.6°N, 97.3°W
Prosper	T4	33.0°N, 96.0°W	Winfield	K7	37.0°N,96.0°W
Stillwater	O1	36.0°N, 97.0°W	Lincoln	NE1	40.8°N, 96.6°W
Goodwell	O2	36.5°N, 101.6°W	Clay Center	NE2	40.5°N, 98.0°W
Lahoma	O3	36.3°N, 98.0°W	North Platte	NE3	41.1°N, 100.7°W
Granite	O4	34.9°N, 99.3°W	Sidney	NE4	41.1°N, 102.9°W
Akron	C1	40.1°N, 103.2°W	Alliance	NE5	42.0°N, 102.0°W
Burlington	C2	39.3°N, 102.2°W	Brookings	S1	44.3°N, 96.7°W
Fort Collins	C3	40.5°N, 105.0°W	Dakota Lakes	S2	44.1°N, 100.0°W
Walsh	C4	37.3°N, 102.2°W	Pine Bluffs	W1	41.1°N, 104.0°W

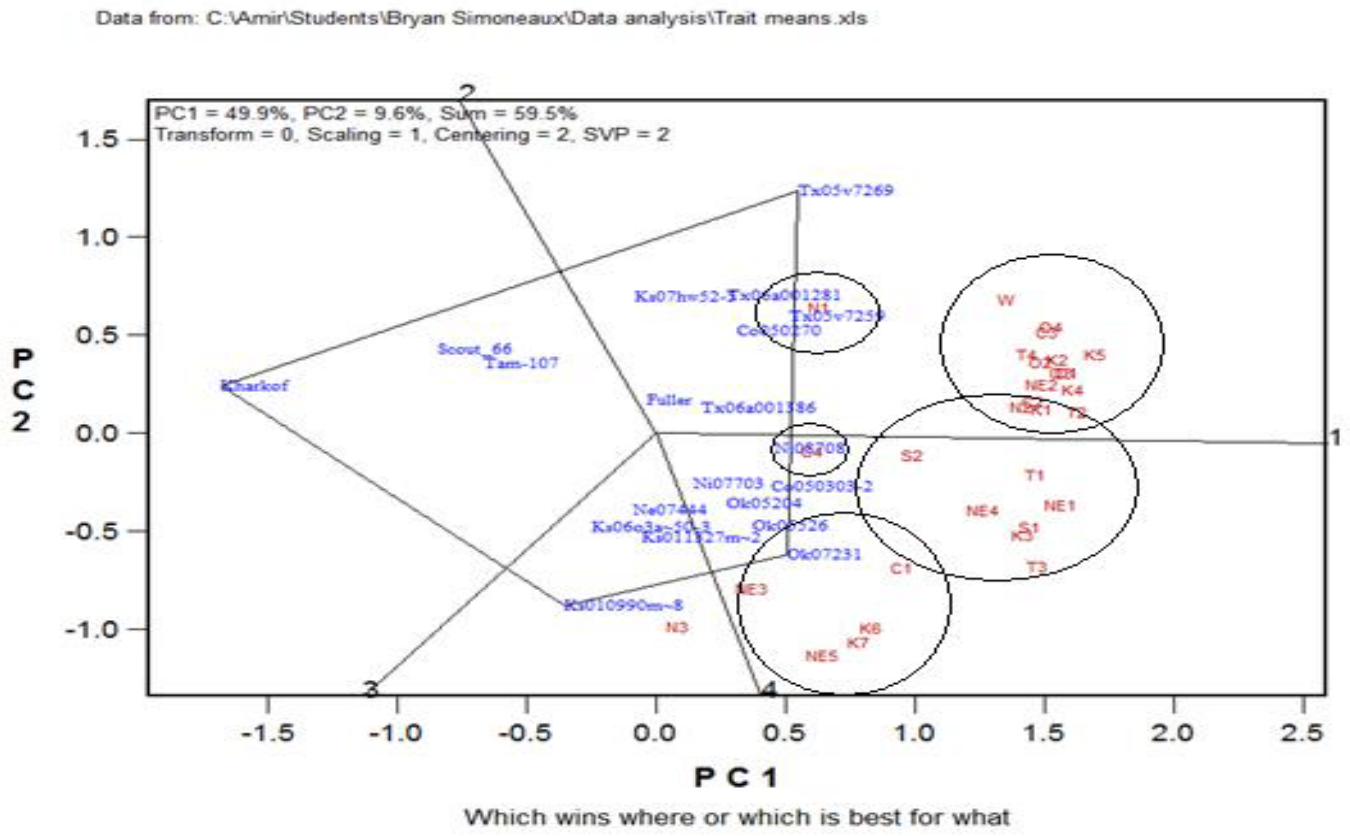

Fig. 3 Yield performance of the Southern Regional Performance Nursery (SRPN) lines done in 2010 across 30 U.S. Locations. (Abbreviations: Clovis Dryland-N1, Clovis Irrigated-N2, Farmington-N3, Bushland dryland-T1, Bushland Irrigated-N2, Chilicothe-T3, Prosper T-4, Stillwater-O1, Goodwell-O2, Lahoma O-3, Granite O-4, Akron C-1, Burlington C-2, Fort Collins C-3, Walsh C-4, Hays K-1, Hutchinson K-2, Salina K-3, Colby K-4, Garden City K-5, Wichita K-6, Winfield K-7, Lincoln NE-1, Clay Center NE-2, North Platte NE-3, Sidney NE-4, Alliance NE-5, Brookings S-1, Dakota Lakes S-2, Pine Bluffs W-1).

The Biplot below for association of ΔP and ΔV with agronomic traits, such as yield, plant height, and days to heading, explained 79.3% of the total variation with 60.8% by PC1 and 18.5% by PC2 (Fig. 4). The biplot shows that Kharkof was the tallest and latest as it is the vertex genotype farthest to the right of the biplot. Scout 66 and TAM 107 were also negatively associated with yield and were among the tallest and latest genotypes. In other studies, early genotypes were shorter and had a reduction in spikelets per head but had a net increase in grains per head due to higher spike fertility (Worland et al., 1998). Genotype OK07231 was the vertex genotype for grain yield based on its position between vertex 3 and 4.

Figures 5 and 6 depict the mean performance and stability of the HRW genotypes for ΔP, and ΔV as well as grain yield, plant height, and days to heading. The genotypes were evaluated for both yield performance and stability across environments. The red arrow points to the values for the different traits; while the blue arrows indicate variability or decreased stability in either direction. The genotypes located to the right of the blue vertical line indicate consistently higher values for traits across all locations.

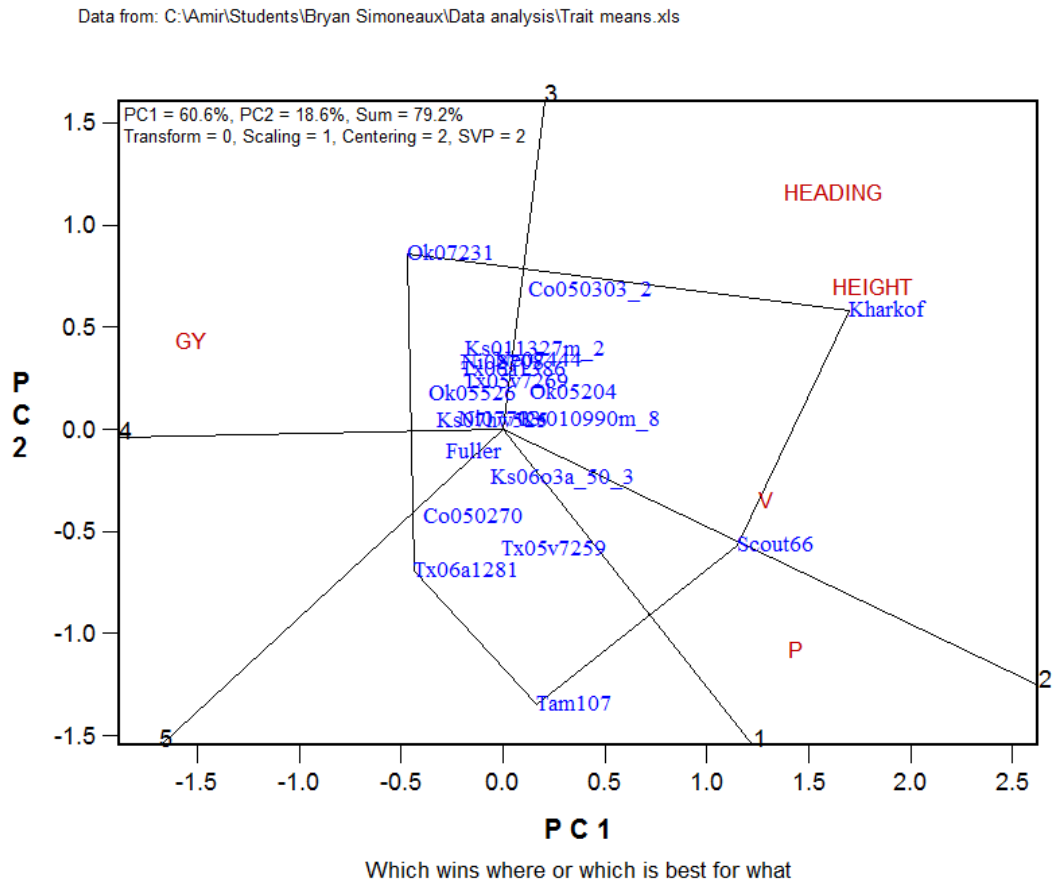

Fig. 4 Association of the main effect of photoperiod (P) and vernalization (V) with yield, height, and heading date for 20 lines representing the 2010 Southern Regional Performance Nursery (SRPN).

The means and stability biplot for ΔP and ΔV classifies the entries based on main effects of photoperiod and vernalization. The biplot explained 100% of total variation with 79.1% explained by PC1 and 20.9% by PC2 (Fig. 5). The cultivars TAM107 and Scout66 ranked the most photoperiod-sensitive in the set. TX05V725 and Kharkov ranked highest for vernalization requirements; whereas the genotype Ok07231 had the lowest ΔV and ΔP. Lines that fell to the right of the ordinate had a high ΔP and/or ΔV. TXo5V725 and Kharkov had the highest ΔV and Scout 66 and TAM 107 had the highest ΔP. Lines projected from the Abscissa approximate standard deviations and are indicative of stability; in addition, the genotypes further away from this line tend to be less stable. OK07231had the lowest ΔP and ΔV and was the most stable genotype in the test.

The means and stability for grain yield, plant height, days to heading as well as ΔP and ΔV are illustrated in Fig. 6. This Biplot explained 79.3% of total variation with PC1 and PC2 explaining 60.8% and 18.5% of the variability, respectively. Genotypes projected from the abscissa approximate standard deviations and are indicative of stability; in addition, the genotypes further away from this line tend to be less stable. Cultivars TAM107 and Scout66 were the least stable for combined traits. Genotypes that fell to the right of the ordinate were generally the tallest, latest, and had higher vernalization and photoperiod response. According to this biplot view, Kharkof was tallest and latest genotype in the set with very poor yield potential and very high vernalization requirement. Cox et al. (1988) compared old and new HRW cultivars and evaluated the genetic improvement in agronomic traits of cultivars released between

1919 and 1987. Included in this study was Kharkof, a cultivar introduced from Russia in the 1900's which we used as a check in our study. They noted that it would be very difficult to separate genetic effects and their interaction effects by any method of estimation on long-term breeding progress. It was noted that it may not be appropriate to use checks such as Kharkof because of its excessive height and later heading compared to modern HRW cultivars. However, Kharkov was used in many studies because it was included as a long-term check in many regional nurseries. Genotypes TX06A1281 and CO050270 were the highest yielding and were among the earliest and shortest with lower vernalization requirement and photoperiod sensitivity. These results confirm that TX06A1281 seemed to be the most adapted genotype based on all five traits measured in this study.

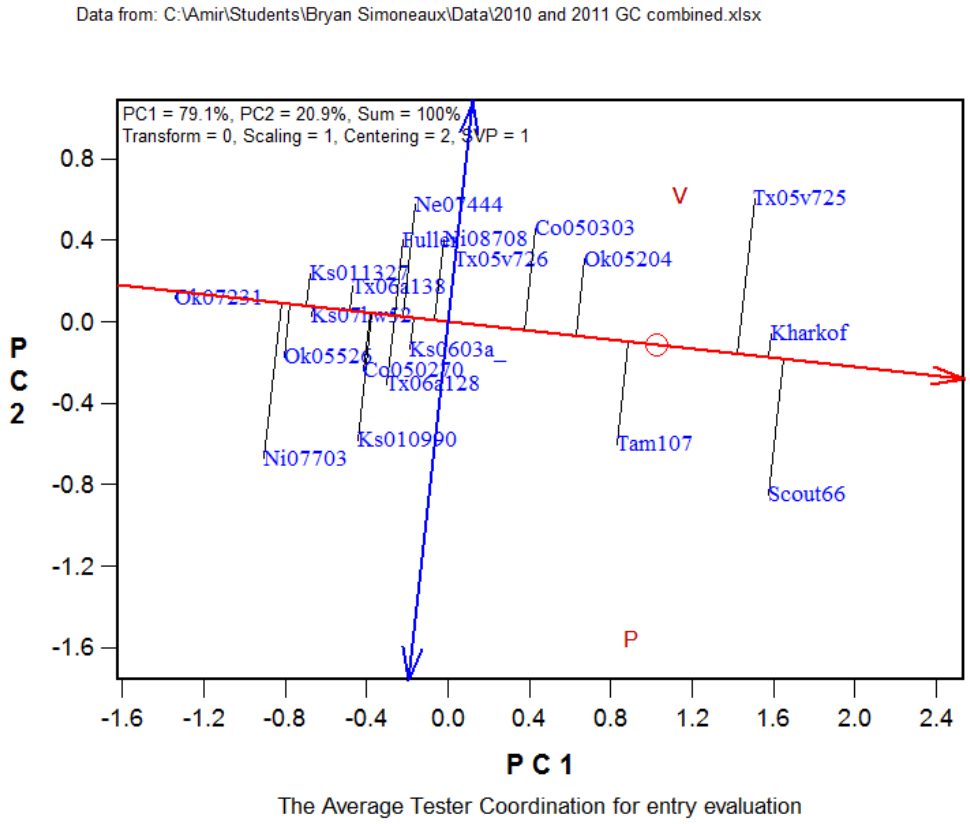

Fig. 5. Average tester coordination view based on photoperiod and vernalization response of 20 HRW lines tested in the growth chamber. Vertical line represents the ordinate, and the horizontal line represents the abscissa. The circle on the abscissa represents the Average environment coordinate. Genotypes that are above the ordinate have higher vernalization sensitivity. Whereas, genotypes which fell below the ordinate have a higher photoperiod sensitivity. The lines to the left of the ordinate are less sensitive to photoperiod and vernalization requirement. . Lines projected from the Abscissa approximate standard deviations and are indicative of stability; in addition, the genotypes further away from the line tend to be less stable.

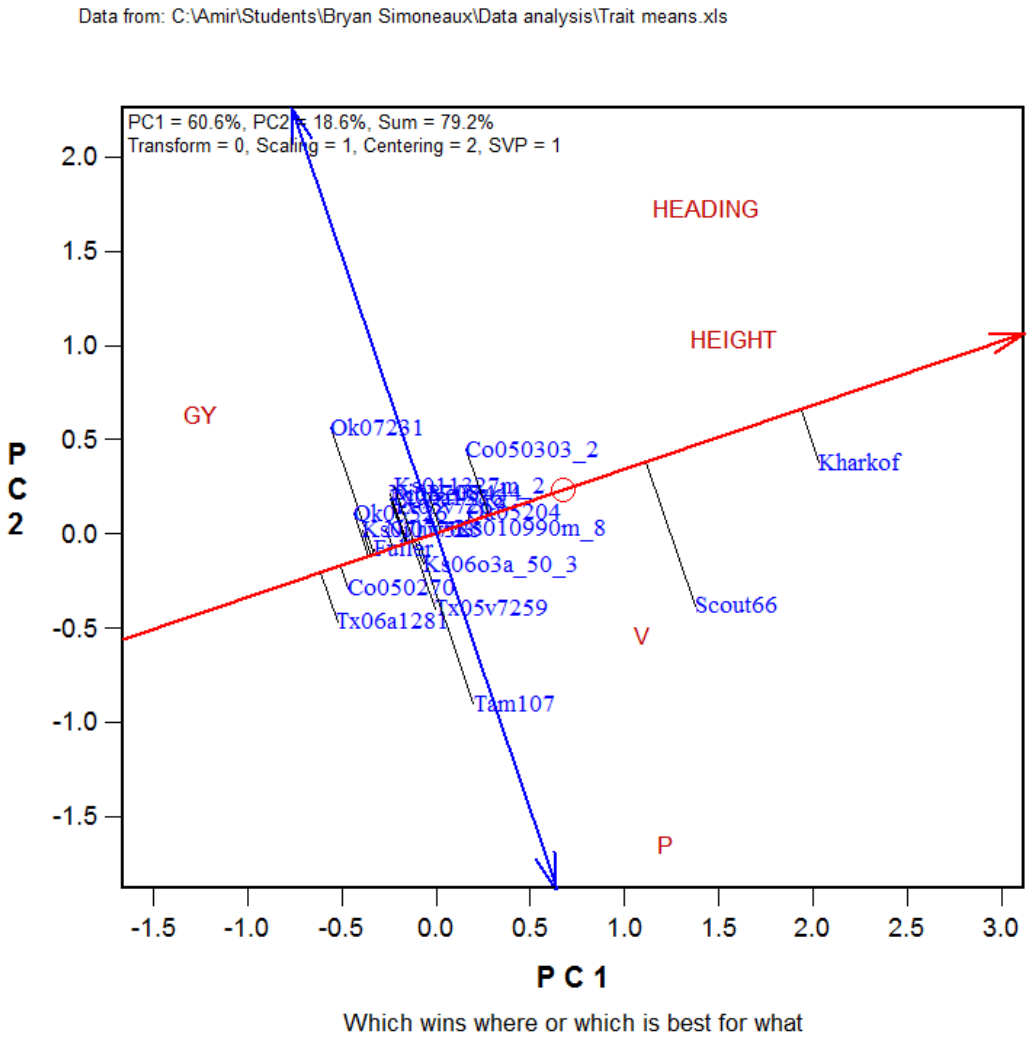

Fig. 6. Average tester coordination view based on the main effect of photoperiod (P) and vernalization (V) of 20 HRW lines tested in the growth chamber in relation to field performance averaged across 30 locations in the 2010 Southern Regional Performance Nursery (SRPN). Vertical line represents the ordinate, and the horizontal line represents the abscissa. The circle on the abscissa represents the Average environment coordinate. Lines projected from the Abscissa approximate standard deviations and are indicative of stability; in addition, the genotypes further away from the line tend to be less stable.

CHAPTER IV

SUMMARY

This study shows that the relationship between photoperiod sensitivity and vernalization requirements are critical in breeding highly adapted hard red winter wheat (HRW) cultivars that perform well across the U.S. Great Plains but remain poorly understood. Furthermore, the current genetic markers for these traits are insufficient to explain all of the variation. A negative correlation was found between yield stability across a broad geographic area, representing 30 U.S. locations, and each of vernalization requirement and photoperiod sensitivity.

Increase in yield potential of U.S. HRW is attributed not only to dwarfing genes, increase in harvest index, and disease resistance but also to lower vernalization requirement and photoperiod insensitivity as shown by the performance and stability of older cultivars such as Kharkof and Scout 66 and newer genotypes in the 2010 Southern Regional Performance Nursery (SRPN). Genotypes such as TX06A1281, which tested PPD-D1 LD insensitive, VRN-A1intermediate-winter and VRN-D3 early winter, seem to perform best in most Texas Environments. Lines such as Kharkof, which tested PPD-D1 LD sensitive, VRN-A1 weak winter and VRN-D3 late winter seem to perform worst in most Texas Environments.

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